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EXAMINING SYNERGISTIC EFFECTS OF TDZ AND TIBA ON ADVENTITIOUS SHOOT INDUCTION IN *DIANTHUS CARYOPHYLLUS* L. LEAF EXPLANTS

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ABSTRACT

The aim of the present investigation was to study the combined effect of cytokinin thidiazuron (TDZ) and auxin-transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) on adventitious shoot induction in leaf explants of carnation (*Dianthus caryophyllus*) cultivar 'Master'. In this study, various concentrations and combinations of TDZ and TIBA were used for induction of adventitious shoots in leaf explants. The explants showed adventitious shoot regeneration (12.33%) on modified Murashige and Skoog's (MS) medium supplemented with 1.0 mg/l TDZ and 1.0 mg/l TIBA while highest frequency of shoot regeneration was observed on MS medium supplemented with 1.0 mg/l TDZ and 0.5 mg/l TIBA (18.33%). Increase in shoot number as well as shoot length was observed with progressive subculturing of shoots. Subculturing of adventitious shoots gave highest average number of shoots (10.22). Shoots were subsequently rooted on MS medium devoid of any phytohormones, supplemented with activated charcoal and rooted plantlets were successfully acclimatized. The combined effects of cytokinin TDZ and auxin transport inhibitor TIBA were found to promote shoot multiplication *in vitro* and prolonged exposure of shoot cultures to TIBA was not found detrimental to shoot growth and multiplication.

KEYWORDS: Carnation, Adventitious Shoots, TDZ, TIBA

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is indigenous to the Mediterranean region. It belongs to the family Caryophyllaceae that has 88 genera and 1750 species. Carnations have been cultivated for over 2000 years ago. Modern varieties of carnations were developed first in France in 1840 (Ali et al. 2008). Carnation is a popularly cultivated cut flower crop throughout the world. In western countries such as USA, it ranks next only to roses in popularity. Carnation is cultivated widely on a large scale in Italy, Spain, Columbia, Kenya, Sri Lanka, India, Canary Islands, France, Holland, Germany and USA (Laurie et al. 1968; Staby et al. 1976; Ruffino et al. 1990; Haouala and Zid 2005).

India with varied climatic zones offers a tremendous scope for its cultivation where farmers cultivate carnations for cut flower market (Mehta et al. 2007; Kumar et al. 2006; Casas et al. 2010). Besides aesthetic value, carnation flowers are considered to be cardiotonic, diaphoretic and alexiteric (Thakur et al. 2002; Kumar et al. 2006). Because of high demand in floriculture market and susceptibility of many cultivars to fungal and viral phytopathogens, there is a pressing need for healthy and true-to-type planting material of carnation cultivars (Sharma and Sharma 2008; Arici and Koc 2009; Kayamori et al. 2012).

Multiplication of commercial carnation cultivars through adventitious shoot regeneration methods, facilitate availability of good, disease-free planting material. Many factors such as genotype, culture medium, type of explant, plant growth hormones, their concentration and combination and environmental factors affect the adventitious shoot induction *in vitro* (Onamu et al. 2003; Pareek et al. 2004; Casanova et al. 2008; Kanwar and Kumar 2009; Casas et al. 2010). Direct regeneration of adventitious shoots in the explants also provides a best and dependable method for the production of large quantities of uniform plantlets in a short period of time.

Adventitious shoot formation for carnation (Dianthus caryophyllus L.) has been previously obtained from petals (Kakehi 1979), axillary buds (Miller et al. 1991), stems (Nugent et al. 1991) and leaves from in vitro and greenhouse grown plants (Van Alvorst et al. 1992), but effects of unconventional plant hormones like auxin-transport inhibitor on in vitro cultures has not yet been studied in carnation. All plants are known to produce auxins and cytokinins *in vivo* and hence, the intrinsic plant hormone levels significantly influence the response of explants to *in vitro* organogenesis. Carnation is considered a difficult plant for *in vitro* shoot induction in explants other than nodal segments. Shiba and Mii (2005) reported the difficulty in organogenesis in most Dianthus species, especially carnation (Nakano and Mii 1992; Kanwar and Kumar 2009).

It is reported that TDZ induces shoot regeneration in many plant species. TDZ is an effective cytokinin that is used for shoot induction in plants where *in vitro* morphogenesis is difficult (Guo et al. 2011). In many reports, thidiazuron is found effective for shoot regeneration in carnation cultivars (Lu 1993). Lu (1993) suggested that thidiazuron is more effective than benzyladenine, kinetin or zeatin for induction of shoot regeneration in carnation (*Dianthus* spp.) and rose (*Rosa* spp.). Thidiazuron at low concentrations (0.002-0.088 mg/l) was effective for propagation and shoot number produced on TDZ containing medium was more than that initiated on the medium containing other cytokinins. Similarly, Casanova et al. (2004) reported that TDZ alone (0.5 and 5.0 µM/l) as well as synergistically with NAA (0.5 and 5.0 µM/l) promoted shoot organogenesis in carnation petals. TIBA (2, 3, 5-triiodobenzoic acid) is an auxin-transport inhibitor, which is used as a plant growth regulator mainly for shoot induction in plants that show high level of intrinsic auxins. Marks and Simpson (1994) found that TIBA promoted shoot proliferation and reduced callus formation in shoot cultures derived from mature trees of *Acer*. Similarly Sugimura et al. (1999) reported that, combination of cytokinin BA or TDZ along with TIBA was effective in adventitious shoot regeneration from leaf explants of mulberry and suggested that TIBA, an auxin-transport inhibitor, is effective in plants that show high level of intrinsic auxins.

Therefore the present investigation aimed at studying combined effects of TDZ and TIBA on leaf explants of carnation *in vitro* and to find their role in induction of adventitious shoots.

MATERIALS AND METHODS

Plant Material

Fresh leaves were obtained from greenhouse grown plants of carnation *Dianthus caryophyllus* L. cultivar 'Master'. The leaves were washed under running tap water for 20 min in a clean beaker for few minutes. The surface sterilization of explants was done in the laminar air flow cabinet using 0.2 per cent Bavistin (Carbendazim, United Phosphorous Ltd., Gujarat, India) solution for 1-3 minutes followed by washing 3 times with autoclaved distilled water. Thereafter, explants were treated with 0.5 per cent sodium hypochlorite solution (4% chlorine available) for 5-15 minutes followed by washing 3 times with sterile distilled water. Washings of sterile distilled water were given to remove any

traces of sodium hypochlorite. Then the leaf explants were cut into 0.5-1.0 cm size sections and inoculated on basal MS medium (Murashige and Skoog 1962). The MS salts (SRL), vitamins (SRL), supplemented with 100 mg/l meso-inositol (SRL), 3% sucrose (HIMEDIA) and 0.8% agar (HIMEDIA) were used as basal medium. The cultures were then incubated in culture room at 16 hr photoperiod at 25-27°C temperature. There were 20 explants in each treatment and each treatment was replicated thrice following completely randomized design.

Induction of Adventitious Shoots

After establishment of surface sterilization procedure, the combined effects of TDZ and TIBA were studied by inoculating leaf explants on MS media supplemented with various concentrations and combinations of the two growth regulators. Leaf explants were inoculated on MS medium supplemented with varying concentrations and combinations of TDZ and TIBA. The cultures were incubated at 25-27°C temperature and 16 hr photoperiod for the induction of adventitious shoot buds. The experiment was replicated thrice and there were 20 explants per replication. Data was recorded for percent shoot induction, average number of shoots and average shoot length.

Subculturing of Adventitious Shoots

For multiplication of *in vitro* raised adventitious shoots, the established microshoots were cut aseptically into 1.0 to 1.5 cm long nodal segments and were inoculated on the best medium found for adventitious shoot induction. In order to multiply microshoots *in vitro*, they were subcultured on fresh medium at the interval of four weeks. Data was recorded for average number of shoots and average shoot length for every subculture passage.

Root Induction in Microshoots and Hardening of *In Vitro* Regenerated Plantlets

In vitro raised microshoots of 2.0-3.0 cm length were cut with sterile blade and scalpel under laminar air flow chamber and were transferred to the rooting medium. The rooting medium consisted of basal MS medium supplemented with 0.2 per cent activated charcoal. The data was recorded as average number of roots per shoot and average root length. After complete development of roots, plantlets were taken out of the culture tubes, taking all the precautions to avoid any damage to its delicate root system. The roots were washed gently under distilled water and adhering medium was removed with the help of soft-haired brush. The plantlet was given a dip in Bavistin solution at 0.01 per cent to avoid fungal contamination. Plastic pots of 10 cm diameter were first filled with sterile cocopeat and sterile sand and the plantlets were carefully placed in the pot, covering the roots with sand. The plants were watered with half strength liquid MS medium at regular intervals for 30 days. Thereafter successfully acclimatized plantlets were transferred to soil.

Statistical Analysis

All the experiments were conducted in a completely randomized design (CRD). The data recorded on different parameters was subjected to analysis of variance using CRD (Gomez and Gomez, 1984) while Arc sine transformation was applied for the data expressed in percentages. The data obtained was analyzed by analysis of variance (ANOVA) test. The experiment was replicated thrice and there were 20 explants per replication. Observations for percent uncontaminated explants, percent shoot induction, shoot length, number of shoots etc. were noted after four weeks of incubation.

RESULTS

Sterilization of Explants

Leaf explants of carnation cv. Master were used for induction of adventitious shoots. For the surface sterilization of leaf explants, Bavistin and sodium hypochlorite (4% chlorine available) were used. The treatment of 0.2 percent Bavistin for 3 minutes and 0.5 per cent sodium hypochlorite for 10 minutes resulted in highest percentage of uncontaminated cultures (87.33%) after four weeks of incubation (Table 1). Decreasing the time of treatment resulted in less percentage of uncontaminated cultures while longer duration of treatment resulted in death of explants.

Adventitious Shoot Induction

After surface sterilization, explants were incubated on MS medium supplemented with varying concentration of TDZ and TIBA. To study the effect of auxin-transport inhibitor TIBA on adventitious shoot induction, various concentrations of TIBA were tried along with varying concentrations of cytokinin TDZ. The results presented in Table 2 show that in the control medium devoid of any growth regulators, there was no shoot induction in leaf explants. However, leaf explants showed response to adventitious shoot regeneration over a narrow range of concentrations of TDZ and in combination with TIBA (Table 2). The initiation of shoot bud development in leaf explants started after 10-12 days of incubation (Figure 1A). In the treatment with TDZ 1.0 mg/l and 0.5 mg/l TIBA, highest per cent (18.33) adventitious shoot induction was observed (Figure 1B) in leaf explants. It may be noted that there was also highest number of adventitious shoots (3.17) regenerated and higher length of shoots (1.53 cm) in the treatment SI_8 as compared to treatment SI_9 which showed lower percentage (12.33) of adventitious shoot induction as well as lower number of shoots (1.90) and length of shoots (0.83 cm). The rest of the treatments failed to produce adventitious shoot induction. Hence the concentration of TDZ at 1.0 mg/l + 0.5 mg/l TIBA was found best for adventitious shoot regeneration from leaf explants of carnation cv. Master as compared to all other treatments. It was observed that concentration as well as combination of plant growth hormones is a key factor in induction of adventitious shoots.

Shoot Multiplication

Out of the different media tried, MS medium supplemented with 1.0 mg/l TDZ + 0.5 mg/l TIBA was selected for further subculturing of shoots to observe the effect of sub-culturing on shoot proliferation. For shoot multiplication, the individual shoots from the shoot clumps were separated and cultured on the same shoot regeneration medium (Figure 1C) till a sufficient rate of shoot multiplication was achieved. There were 20 microshoots in each subculture replicated five times. The number and length of microshoots incresed linearly throughout the subculture passages. Each microshoot produced highest number of shoots (10.20) in third subculture with an average shoot length of 3.90 cm (Table 3) (Figure 1D).

Root Induction and Hardening of *In Vitro* Raised Plantlets

The adventitious microshoots measuring 1.5-2.0 cm in length were isolated and excised for transfer to root induction medium consisting of MS basal medium supplemented with 0.2% activated charcoal to get complete plantlets. Root initiation started generally after 10-15 days in culture and in four weeks, profuse roots were observed (Figure 1E). After complete development of roots, plantlets were taken out of the culture tubes, taking all the precautions to avoid any damage to its delicate root system. Each plantlet was given a dip in Bavistin solution at 0.01 per cent to avoid fungal

contamination. Plastic pots of 10 cm diameter were first filled with 1/3 sterile cocopeat and 2/3 sterile sand and the plantlets were carefully placed in the pot (Figure 1F). About 86% survival rate was observed in transferred plantlets during acclimatization process. The acclimatized plantlets were successfully transferred to soil after a month.

DISCUSSIONS AND CONCLUSIONS

Direct regeneration of adventitious shoots on the explants provides a best and dependable method for the production of large quantities of uniform plantlets in a short period of time. The use of leaf and stem explants has not been successful for induction of adventitious shoots in the previous studies (Miller et al. 1991) largely due to explant senescence in the presence of benzyladenine, kinetin and, to a lesser extent, zeatin. In the present study, explants cultured on MS medium supplemented with different concentrations of TDZ and TIBA showed adventitious shoot regeneration over a narrow range of treatments, where most of the treatments failed to respond to induction of adventitious shoot bud in leaf explants of carnation cv. Master. Also TDZ alone did not produce any adventitious shoots in the present study.

Lu (1993) reported that Thidiazuron at low concentrations (0.002-0.088 mg/l) was effective for propagation and shoot number produced on TDZ containing medium was more than that initiated on the medium containing other cytokinins. Kanwar and Kumar (2009) reported highest average number of shoots observed in leaf explant derived callus on MS medium supplemented with 2.0 mg/l TDZ and 1.0 mg/l IAA in carnation. However highest frequency of adventitious shoots were obtained from leaf base explants in Fea and Rossitza cultivars of carnation on MS medium supplemented with 0.9-mg/l BAP and 0.9 mg/l NAA (Iantcheva et al. 2005) while highest leaf explant response, number of shoots (15.30), and shoot length (6.75 cm) was recorded in response to a combination of 2.5 µM BA and 0.5 µM NAA (Varshney et al. 2013). Therefore it can be concluded that response of explants to *in vitro* adventitious shoot induction depends on concentration and combination of growth hormones, as well as more importantly, genetic makeup of carnation cultivar.

Sugimura et al. (1999) reported that TDZ in combination with TIBA promoted adventitious shoot induction in leaf explants of Mulberry, whereas TIBA in particular was found to have stimulatory effect in shoot induction. In the present investigation aimed to study combined effects of TDZ and TIBA on leaf explants, adventitious shoot induction was observed on MS medium supplemented with 1.0 mg/l TDZ and 0.5 mg/l TIBA in carnation cv. Master that is the first report of successful use of auxin-transport inhibitor TIBA along with TDZ for adventitious shoot regeneration in carnation with reference to the available literature. The MS medium supplemented with 0.2 per cent activated charcoal, devoid of any growth regulators resulted in highest percentage of rooting of *in vitro* raised control as well as selected shoots of carnation cv. Master with highest number of roots and highest root length. Similarly, Kanwar *et al.* (2007) reported rooting on half MS medium supplemented with 0.05 per cent activated charcoal in *Robinia pseudoacacia* L. Similar results were reported (Kumar 2008) for *in vitro* rooting in carnation shoots on half strength MS medium supplemented with 2.0 mg/l IBA and 0.2 per cent activated charcoal.

In the present investigation, adventitious shoot induction was observed on MS medium supplemented with 1.0 mg/l TDZ and 0.5 mg/l TIBA (SI_8) as well as with 1.0 mg/l TDZ and 1.0 mg/l TIBA (SI_9) in carnation cv. Master. However it was observed that, percent shoot induction, number of adventitious shoots induced and shoot length was significantly reduced on increasing concentration of TIBA, while concentration of TDZ was kept the same. It was found that TDZ alone at concentrations of 0.5 mg/l (SI_2) and 1.0 mg/l (SI_7) failed to induce adventitious shoots in leaf explants

whereas low concentration of TDZ was found detrimental to adventitious shoot induction even in presence of TIBA (SI_4 to SI_6). Only when TIBA was supplied at half the concentration of TDZ, significant shoot induction was observed (Table 2). Although percent shoot induction in explants was low, shoots showed high rate of multiplication when multiplied on the same (SI_8) medium. The number of shoots and shoot length, increased with each subculture on the same adventitious shoot induction medium and highest numbers of shoots were obtained in third subculture. Significant increase in number of shoots and shoot length was observed with each subculture passage (Table 3) where number of shoots and shoot length almost doubled from first subculture to third subculture. Thus the synergistic effect of TIBA and TDZ may be seen for the first time in carnation where TIBA was found to have stimulatory effect for direct shoot induction without the callusing phase. The combined effect of cytokinin TDZ and auxin transport inhibitor TIBA was found very good for shoot multiplication *in vitro* and prolonged exposure of shoot cultures to TIBA was not found detrimental to shoot growth and multiplication.

REFERENCES

1. Ali A, Afrasiab H, Naz S, Rauf M, Iqbal J (2008) An efficient protocol for *in vitro* propagation of carnation (*Dianthus caryophyllus*). Pak J Bot 40(1): 111-121
2. Arici S, Koc N (2009) Regeneration and Agrobacterium-mediated transformation studies in carnation (*Dianthus caryophyllus* L. cv. Turbo). African Journal of Biotechnology 8 (22): 6094-6100
3. Casanova E, Moysset L, Trillas M (2008) Effect of agar concentration and vessel closure on the organogenesis and hyperhydricity of adventitious carnation shoots. Biol Plantarum 52(1): 1-8
4. Casanova E, Valdes E, Fernandez B, Moysset L, Trillas M (2004) Levels and *in situ* localization of endogenous cytokinins in thiadizuron induced shoot organogenesis in carnation. Journal of Plant Physiology 161(1): 95-104
5. Casas J, Olmos E, Piqueras A (2010) *In vitro* propagation of carnation (*Dianthus caryophyllus* L.) In: Protocols for In Vitro Propagation of Ornamental Plants, Methods in Molecular Biology 589: 109-116
6. Guo B, Abbasi B, Zeb A, Xu L, Wei Y (2011) Thidiazuron: A multi-dimensional plant growth regulator. African Journal of Biotechnology 10(45): 8984-9000
7. Haouala F, Zid E (2005) Differentiation of shoot buds from internodal callus and plant regeneration in carnation (*Dianthus caryophyllus* L.). Advances in Horticultural Science 19(4): 187-192
8. Iantcheva A, Vlahova M, Atanassova B, Atanassov A (2005) Plant regeneration via direct organogenesis and somatic embryogenesis of two Bulgarian spray carnation cultivars. Biotechnol & Biotechnol Eq 15-19
9. Kakehi M (1979) Studies on the tissue culture of carnation. V. Induction of redifferentiated plants from the petal tissue. Bull Hiroshima Agric Coll 6: 159-166
10. Kanwar J, Kumar S (2009) Influence of growth regulators and explants on shoot regeneration in carnation. Hort Sci (Prague) 36(4): 140–146
11. Kanwar J, Kumar S (2009) Influence of growth regulators and explants on shoot regeneration in carnation. Hort Sci 36:140-146

12. Kanwar K, Bhardwaj A, Raj Deepika, Sharma D (2007) *Robinia pseudoacacia* Linn. Tree and Forestry Science and Biotechnology 1(1): 74-80

13. Kayamori M, Sasaki J, Matsui R, Shinmura A, Horita A, Satou M (2012) First report of downy mildew of carnation caused by *Peronospora dianthicola* in Japan. Journal of General Plant Pathology 78(5): 364-367

14. Kumar A, Verma A, Singh S, Raghava S, Kumar P (2006) *In vitro* shoot regeneration from leaf segments of carnation (*Dianthus caryophyllus* L.) via indirect organogenesis. Plant Cell Biotechnology and Molecular Biology 7: 65-68

15. Kumar J (2008) *Argobacterium* mediated transformation studies for insect resistance in *Dianthus caryophyllus* L. Dissertation, Dr Y S P University of Horticulture and Forestry

16. Laurie A, Kiplinger D, Nelson K (1968) Commercial Flower Forcing. McGraw Hill Co. pp 3-67

17. Lu C (1993) The use of thidiazuron in tissue culture. In Vitro Cell Dev Biol-Plant 29(2): 92-96

18. Marks T, Simpson S (1994). Factors affecting shoot development in apically dominant *Acer* cultivars *in vitro*. Hortic Sci 69: 537-543

19. Mehta R, Sharma S, Nath A (2007) *In vitro* selection and biochemical characterization of carnation (*Dianthus caryophyllus* L.) callus tolerant to *Alternaria dianthi.* Indian Journal of Plant Physiology 12(2): 120-126

20. Mii M, Buiatti M, Gimelli F (1990) Carnation In: Amirato et al. (eds) Handbook of Plant Cell Culture, McGraw Hill, New York, USA, pp 284-318

21. Miller R, Kaul V, Hutchinson J, Richards D (1991) Adventitious Shoot Regeneration in Carnation (*Dianthus caryophyllus*) from Axillary Bud Explants. Ann of Bot 67: 35-42

22. Murashige T, Skoog I (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum 15: 473-497

23. Nakano M, Hoshino Y and Mii M. 1994. Adventitious shoot regeneration from cultured petal explants of carnation. *Plant Cell, Tissue and Organ Culture* **36**:15-19.

24. Nugent G, Wardley-Richardson T, Lu S (1991) Plant regeneration from stem and petal of carnation (*Dianthus caryophyllus* L.). Plant Cell Rep 10: 477-480

25. Onamu R, Obukosia S, Musembi N, Hutchinson M (2003) Efficacy of thidiazuron in *in vitro* propagation of carnation shoot tips: influence of dose and duration of exposure. Afr Crop Sc 11(2): 125-132

26. Pareek A, Kantia A, Kothari S (2004) *In vitro* cloning of ornamental species of *Dianthus*. Indian J Biotechnol 3: 263-266.

27. Ruffino B, Massbo F, Curir P, Carla D (1990) Adventive regeneration in cell colonies of carnation. Acta Horticulturae 280: 173-176.

28. Sharma S, Sharma N (2008) Carnation diseases and their management- a review. Agric Rev 29(1): 11-20

29. Shiba T, Mii M (2005) *Agrobacterium tumefaciens*– mediated transformation of highly regenerable cell suspension cultures in *Dianthus acicularis*. Journal of Horticultural Science and Biotechnology 80: 393–398

30. Staby G, Robertson J, Kiplinger D, Conover C (1976) Proceedings in National Floriculture Conference Commod, Handl. Colombus, Ohio pp 1-77

31. Sugimura Y, Adachi T, Kotani E, Furusawa T (1999) Efficient induction of shoot organogenesis from leaves of Mulberry seedlings using 2,3,5-triiodobenzoic acid. Plant Biotechnology 16(2): 123-127

32. Thakur M, Sharma D, Sharma S (2002) *In vitro* selection and regeneration of carnation (*Dianthus caryophyllus* L.) plant resistant to culture filtrate of *Fusarium oxysporum* f. sp. *dianthi*. Plant Cell Reports 20: 825-828

33. Van Alvorst A, Koehorst H, Bruinsma T, Jansen J, Custers J, De Jong J, Dons J (1995) Adventitious shoot formation from *in vitro* leaf explants of carnation (*Dianthus caryophyllus* L.). Scientia Horticulturae 51: 223-235

34. Varshney A, Anis M, Aref I (2013) Potential role of cytokinin–auxin synergism, antioxidant enzymes activities and appraisal of genetic stability in Dianthus caryophyllus L.—an important cut flower crop. In Vitro Cellular & Developmental Biology – Plant 49(2): 166-174

35. Yadav M, Gaur A, Garg G (2003) Development of suitable protocol to overcome hyperhydricity in carnation during micropropagation. Plant Cell, Tissue and Organ Cult 72: 153-156

APPENDICES

Table 1: Effect of Different Treatment Durations of Bavistin and Sodium Hypochlorite Solution on Surface Sterilization of Leaf Explants (*Figures in Parentheses are Arc Sine Transformed Values)

Treatment (X)	0.2% Bavistin Treatment Duration (Minutes)	0.5% (v/v) Sodium Hypochlorite Treatment Duration (Minutes)	Per Cent Uncontaminated Explants
X_1	1.00	5.00	25.33(30.22)*
X_2	2.00	5.00	35.33(36.47)
X_3	3.00	5.00	40.50(39.43)
X_4	1.00	10.00	59.67(50.58)
X_5	2.00	10.00	76.00(60.67)
X_6	3.00	10.00	87.33 (69.17)
X_7	1.00	15.00	61.67(51.75)
X_8	2.00	15.00	70.67(57.22)
X_9	3.00	15.00	75.00(60.00)
$CD_{0.05}$			1.35
SE±			0.64

Table 2: Effect of Different Concentrations of TDZ in Combination with TIBA on Induction of Adventitious Shoots from Leaf Explants of Carnation cv. Master (*Figures in Parentheses are Arc Sine Transformed Values)

Treatment Sr. No. SI	Phytohormones (mg/l)		Per Cent Shoot Induction	Average No. of Shoots	Average Shoot Length (cm)
	TDZ	TIBA			
SI_1	0.0	0.0	0.00(0.00)*	0.00	0.00
SI_2	0.5	0.0	0.00(0.00)	0.00	0.00
SI_3	0.5	0.5	0.00(0.00)	0.00	0.00
SI_4	0.5	1.0	0.00(0.00)	0.00	0.00

Table 2: Contd.,

SI_5	0.5	1.5	0.00(0.00)	0.00	0.00
SI_6	0.5	2.0	0.00(0.00)	0.00	0.00
SI_7	1.0	0.0	0.00(0.00)	0.00	0.00
SI_8	1.0	0.5	18.33(4.28)	3.17	1.53
SI_9	1.0	1.0	12.33(3.51)	1.90	0.83
SI_{10}	1.0	1.5	0.00(0.00)	0.00	0.00
SI_{11}	1.0	2.0	0.00 (0.00)	0.00	0.00
SE±			0.06	0.09	0.04

Table 3: Effect of Subculturing of Adventitious Shoots

Subculture Passage	Average No. of Shoots	Average Shoot Length (cm)
I	5.40	2.25
II	7.80	3.04
III	10.20	3.90
SE±	0.87	0.23

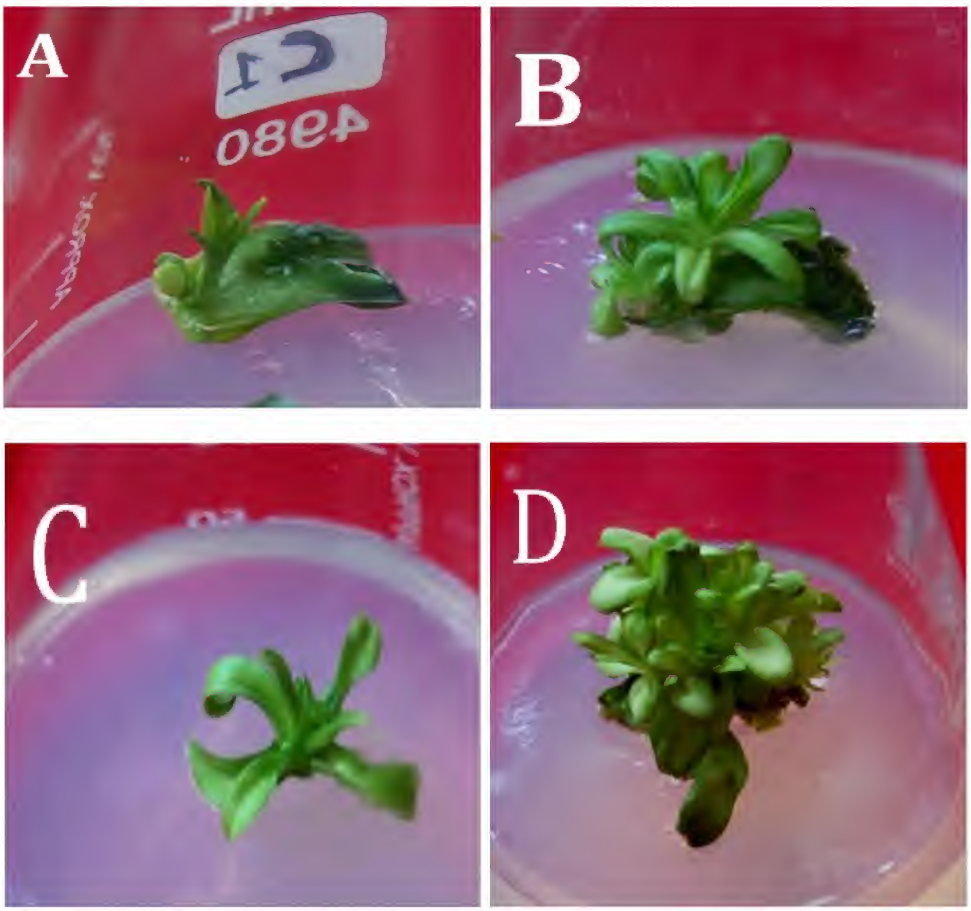

Figure 1A: A: Induction of Adventitious Shoot Buds in Leaf Explants Cultured on MS Medium Supplemented with 1.0 mg/l TDZ and 0.5 mg/l TIBA after 10 Days of Incubation, B: Development of Adventitious Shoots in Leaf Explants after 4 Weeks of Incubation on MS Medium Supplemented with mg/l TDZ and 0.5 mg/l TIBA, C: Inoculated Microshoot on Shoot Multiplication Medium, D: Shoot Multiplication 3 Subcultures on Shoot Multiplication Medium, E: Rooting of Microshoot on Medium Supplemented with Activated Charcoal, F: Hardening of *In Vitro* Regenerated Plantlet